European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Lactoferrin Quantification in Human Colostrum from Indian Mothers' and its Association with Several Maternal and Neonatal Parameters

Article History:

Name of Author:
Aishwarya Das[1], **Sukanya Chakravorty[2], Deepa Mishra[3], S.P Mishra[4], P.S. Upadhyaya[5], Muskan Bharti[6], Shalini Mishra[7]

Affiliation:

[1]Research Scholar, [2] Assistant Professor, Department of Home Science

[3&4]Professor, Department of Prasuti Tantra and Department of Clinical Biochemistry

[5]Associate Professor, Department of Kaumarbhritya/Balroga

[6&7]Research Scholar, Department of Clinical Biochemistry Banaras Hindu University

**** co-responding author**

Received: 11-11-2025
Revised: 26-11-2025
Accepted: 16-12-2025
Published: 23-12-2025

Abstract: Lactoferrin is an iron binding glycoprotein found in several body fluid including saliva, tears, bile, synovial fluids, plasma and especially in breast milk and even the human colostrum contains highest quantity of it. It has some proven role in protecting infants from neonatal sepsis and GI related issues which are the most dominant cause of neonatal mortality. There are several factors which influence the quantity of Lactoferrin in human colostrum among which neonatal infection, gestational age, parity, allergic response and maternal diet impacts in a positive way. The present study aims to quantify the amount of lactoferrin present in the human colostrum and to find out the co-relation between several maternal and neonatal parameters. A total of 5 ml. colostrum was collected from the lactating mothers till day 4 of delivery based on the availability and secretion of colostrum. The study was conducted in the Indian Medicine Wings of Sir Sunderlal Hospital of Banaras Hindu University, Varanasi. A total of 150 mothers were included in the study among them 195 mothers were able to produce sufficient colostrum and the sample was collected from them through manual process by a trained person. After collection the samples are immediately stored in the -20-degree freezer for further use. The samples were cold centrifuged twice at 4 degrees centigrade in 3500g to separate the interfering substances then Enzyme Linked Immunosorbent Assay (ELISA) was performed. Nutrient analysis was done using 24-hour dietary re-call method. Other data was collected using a self-structured questionnaire by interview schedule method. Results were analyzed in SPSS software (27 version) and expressed in percentage and association was established using a co-relation test. A p value less than 0.05 was considered to be statistically significant. The study shows a positive co-relation between iron and fat intake with the concentration of colostrum ($p<0.05$). Gestational age of the mothers also a contributing factor. The present study suggests that lactoferrin concentration varies due to several maternal and neonatal factor. Although more research required to establish or support and refute the factors effecting the lactoferrin concentration.

Keywords: Human colostrum, lactoferrin, maternal diet, ELISA.

INTRODUCTION

Lactoferrin is an immunologically active molecule which protects the infants from several neonatal diseases both directly and indirectly and its concentration varies from colostrum to mature milk (1). Lactoferrin acts by binding with the lipoprotein part of the bacterial cells which prevents iron uptake by the bacteria and inhibit their growth (2). It is an iron-binding glycoprotein which confers its multiple benefits including anti-inflammatory, pre-biotic, cellular proliferation and differentiation (3). Lactoferrin is considered to be the second most important protein which acts as a prebiotic and influence the development of the infants by impacting the gut microflora (4). There are several reasons behind the death of neonates among which sepsis tends to be the most common, affecting the premature and low birth weight mostly and lactoferrin even supplementation with bovine one is prove to be effective in the prevention of this life threating situation (5). Along with this, the antimicrobial component has proven to be effective against the enterocolitis among neonates, this acts by inhibiting the growth of the micro-organism responsible for the condition (6). This highly impactful whey protein, has been proved to enhance the mucosal cell growth and maturation of the duodenal part of the small intestine, however several studies based on laboratory shows this impact (7). There are several factors that deals with the concentration of in human milk among which neonatal infection, parity, maternal nutritional status, gestational age, allergic response, stage of lactation matters the most (8). Maternal malnutrition or infection along with iron status buffers the concentration of lactoferrin, even the stage of lactation specifically for first 2 month impact the same (9). Among all the factors, nutrients present in maternal diet including energy, protein, vitamin A are associated with its concentration and intake of egg has proven impact (10).

The present study aimed to quantify the lactoferrin in human colostrum and explore the impact of various maternal and neonatal parameters on the concentration of it.

Materials and Methodology:

Ethical permission: Ethical permission was obtained from Institutional Ethical Committee and Clinical Trial Registry of India. CTRI/2024/06/068448 and ECR/226/Indt/UP/2014/RR-22 .

Study area: Prasuti ward, Indian Medicine wings of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi.

Case registration: A total of 195 lactating mothers were included in the present study through simple random sampling methods.

Materials used: A self-structured questionnaire was used to collect the primary data from mothers. Secondary data was collected for the information related to neonates from the hospital registers. Human Lactoferrin Kit (Abkine) was used to determine the concentration in colostrum.

Methods used: Interview schedule method was used to collect the data. Nutrient analysis was performed using 24- hour dietary recall method. Calculation for carbohydrates, protein, total energy, fat and iron were done by using IFCT-2024. Colostrum was collected about 5ml. from mothers post-delivery up to 4 days. It was collected through manual ejection and performed by a technical person. Immediately the samples were stored in -20-degree freezer. Lactoferrin was analyzed using Sandwich ELISA methods, which is described below.

Sample Preparation:

Human colostrum samples were centrifuged twice at 4-degree centigrade 3500g to remove the interfering substances. After that sandwich ELISA method was performed to determine the concentration of lactoferrin in the samples.

Standard preparation:

A total of 5 standard was prepared by serially diluting the standard solution. If the samples generate values higher than the highest standard, then sample dilution is required and even repeating the assay might be necessary.

Wash Buffer Preparation:

wash buffer was prepared in a ratio of 1: 30. After calculation 9 ml. of wash buffer was required to perform the assay for 1 complete plate.

Procedure:

All the standard and reagents were prepared before starting the assay procedure. Diluted standard of 50ul were added in the standard well of the ELISA kit. 40 ul of sample diluent were added to the testing samples and then 10ul of testing samples are added to each well. Whereas blank well does not contain anything. The plate was covered and incubated for 45 minutes at room temperature. Each well was aspirated and washed using a wash buffer which repeated for four times for a total of five times, 1-3 minutes per time. Removal of liquid was essential for good performance. Further any wash buffer was removed by aspirating or decanting, followed by inverting the plate and blot it against clean paper towels. After that, 50 ul of HRP- Conjugated detection antibody was added to each of well, except for the blank, plate was covered and incubated for 30 minutes at 37-degree C. The wash process was repeated for five times. After this stage, Chromogen A and B solution was added to each of the well in an amount of 50 ul each, gently mixed and incubated for 15 minutes at 37 degree C in a dark cool place. 50ul of stop solution was added to each well to stop the reaction. The color of the solution changed from blue to yellow. Thorough mixing was required to ensure proper reaction otherwise gently tap the plate. Optical density was measured 450 nm using a microtiter plate reader within 15 minutes.

Dietary Assessment:

The respondents were asked to recall the foods she has consumed one day before the delivery and standard household measurement was used to bring accuracy in the calculation. Then each nutrient was calculated manually and to validate Ntuitive application was used. Dietary assessment was performed for Carbohydrates, protein, fats, total energy and iron.

Statistical Analysis:
Collected data was analyzed using SPSS software version 27. Result was prepared in percentage form and association was established using co-relation method. A p value less than 0.05 was considered as statistically significant. 95 % Confidence Interval was taken.

RESULTS

Table 1: Maternal and Neonatal Information:

Maternal & Neonatal Characteristics	Total number (N)	Percentage (%)
Age		
18-22	16	8
23-27	76	38.9
28-32	86	44.1
33-37	16	8.2
38-42	01	0.5
43 & above	00	00
Parity		
Nulli parity	122	62.5
Multi parity	73	37.4
Gravida		
Primi Gravida	92	47.1
Multi gravida	103	52.9
Mode of Delivery		
SVD	34	17.4
C-section	161	85.5
Gestational age		
Preterm	19	9.7
Full term	175	89.7
Post term	1	0.5
Neonatal birthweight		
LBW	32	16.4
Normal weight	163	83.5
Presence of illness		
Fever	05	2.5
Diarrhea	04	2.0
Birth asphyxia	10	5.1
Hyperbilirubinemia	12	6.1

The above-mentioned table provides the general information about the respondents, where it is clear that most of the mothers were in the age group of 28-32 years and 62.5% of mothers were nulliparous. 85.5% of the respondents undergone C-section and 89.7% of the mothers delivered at their full term that is between 37-39 weeks. While 83.5% of the newborn were having normal birth weight that is 2.5-3.5kgs. 6.1% and 5.1 % of the new born were having hyperbilirubinemia and birth asphyxia at birth (table 1)

Table 2 shows the nutritional information of the mothers. The average Body Mass Index of them was 23 kg/m² which lies within the normal level. Mean haemoglobin level was 10.8 which predicts the cases of moderate anaemia as during pregnancy RBC concentration drops. Blood Glucose level was normal. Among the nutrients, Carbohydrates and fat consumption were found to be high among the mothers according to RDA, whereas protein consumption was low. Iron consumption was also lower among them.

Table 2: Maternal Nutritional Information

Nutritional parameters	Mean	Std. deviation
Height (cm.)	156.9	4.9
Weight (kg.)	56.7	9.6
BMI (kg/m^{2})	23.0	3.6
Hemoglobin(g/dl)	10.8	1.2
Blood glucose(g/dl)	88.91	10.7
TSH (mIU/L)	2.54	1.7
Carbohydrate intake(gm/d)	278.1	80.2
Protein intake(gm/d)	52.9	12.2
Fat intake(gm/d)	49.71	2.99
Calorie intake(kcal/d)	1655.2	312.3
Iron intake(mg/d)	14.1	2.9

Figure 1: Frequency Distribution of Food Groups

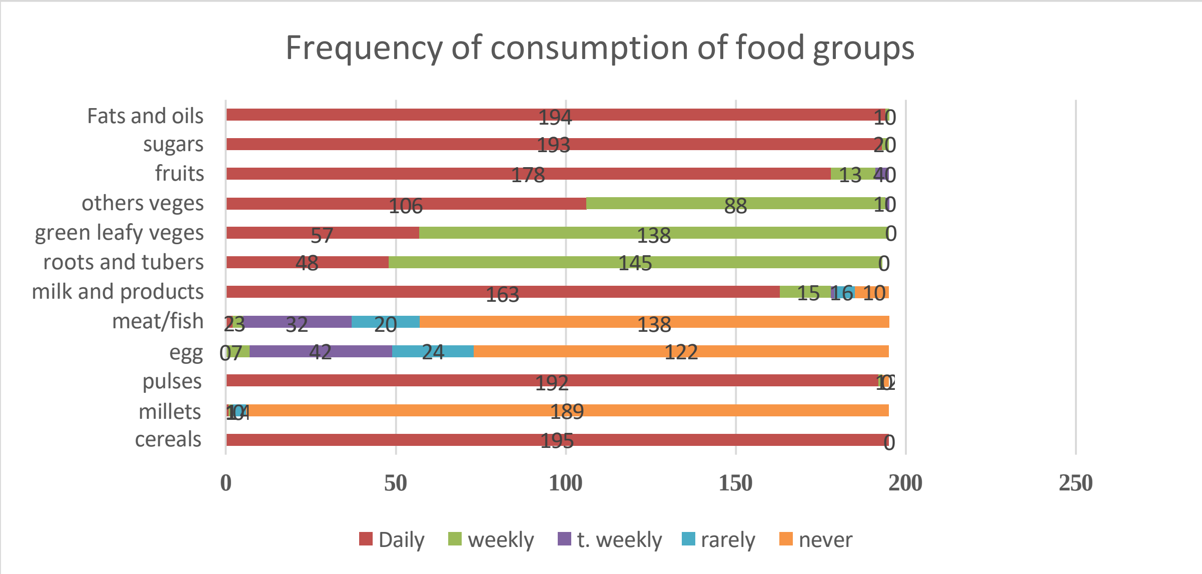

Figure 1 depicts the Frequency of consumption of all of the food groups, where it is found that cereal products were consumed by the respondents daily whereas consumption of millets such as jowar, bajra, ragi etc were very low among them. As most of the mothers were vegetarian due to their cultural believes, consumption of egg or fish and chicken were very negligible. 57 mothers were consuming green leafy vegetables daily. Milk was taken by the mothers on a daily basis (163). 178 of the mothers were taking a whole fruit on a daily basis. Sugar and oil consumption were found to be on a daily among all the participants. The entire figure denotes that mothers were aware of consuming each food groups to maintain a good health.

Table 4: Average lactoferrin concentration (day wise):

Post-partum days	Mean Lactoferrin concentration(ug/ml)	Total no. of mothers (N)	Percentage (%)
Day 1	00	00	00
Day 2	19.16	15	17.8
Day 3	17.30	36	42.8
Day 4	16.60	33	39.2

Table 4 shows the average concentration Lactoferrin in human colostrum. It is clearly seen that concentration of lactoferrin has decreased with the post-partum days. The highest concentration found in the colostrum collected in day 2. 42% of the respondents donated their colostrum on day 3 after delivery. Zhou et al., conducted a study in 2025 which has also shown the longitudinal change in the lactoferrin concentration among the lactating mothers.

Table 5 provides the information regarding the significant co-relation between the nutrients and also with the lactoferrin concentration. It is found that lactoferrin concentration is significantly associated with the dietary iron and fat intake ($p<0.05$). no significant relation was found with carbohydrate or protein intake. As lactoferrin is an iron binding protein.

Table 5: Co-relation with maternal nutrient intake and Lactoferrin concentration:

		Lactoferrin	Energy	Protein	Carbohydrates	Iron	fats
Lactoferrin	Pearson Correlation	1	.045	.005	.090	-.130	-.182*
	Sig. (2-tailed)		.534	.944	.209	.071	.011
	N	195	195	195	195	195	195
Energy	Pearson Correlation	.045	1	.694**	.804**	.377**	.272**
	Sig. (2-tailed)	.534		.000	.000	.000	.000
	N	195	195	195	195	195	195
Protein	Pearson Correlation	.005	.694**	1	.587**	.365**	.321**
	Sig. (2-tailed)	.944	.000		.000	.000	.000
	N	195	195	195	195	195	195
Carbohydrates	Pearson Correlation	.090	.804**	.587**	1	.344**	-.016
	Sig. (2-tailed)	.209	.000	.000		.000	.825
	N	195	195	195	195	195	195
Iron	Pearson Correlation	-.130	.377**	.365**	.344**	1	.176*
	Sig. (2-tailed)	.071	.000	.000	.000		.014
	N	195	195	195	195	195	195
Fats	Pearson Correlation	-.182*	.272**	.321**	-.016	.176*	1
	Sig. (2-tailed)	.011	.000	.000	.825	.014	
	N	195	195	195	195	195	195
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

A study conducted in 2021 by Lijun et al, also found the significant association with the maternal nutrition and lactoferrin concentration. Huang et al., in his study in the year 2025 also mentioned about the role of iron saturation in the lactoferrin concentration in human milk.

Table 6: Co-relation with parity and lactoferrin concentration:

		Lactoferrin	Parity
Lactoferrin	Pearson Correlation	1	.091
	Sig. (2-tailed)		.205
	N	195	195
Parity	Pearson Correlation	.091	1
	Sig. (2-tailed)	.205	
	N	195	195

Table 6 depicts the non-existence of any significant co-relation between parity and lactoferrin concentration because the present study consists of mothers with higher percentage in nulliparity where as another study conducted in 2020 by Mohammad and Pungestuti, found a positive co-relation between the variables.

Table 7: Co-relation with gestational age and lactoferrin concentration:

		Lactoferrin	Duration of gestation
Lactoferrin	Pearson Correlation	1	.158*
	Sig. (2-tailed)		.027
	N	195	195
Duration of gestation	Pearson Correlation	.158*	1
	Sig. (2-tailed)	.027	
	N	195	195
*. Correlation is significant at the 0.05 level (2-tailed).			

Table 7 shows the significant positive co-relation between the gestational age and the lactoferrin concentration. A study conducted in the year 2016 by Villavicencio et al., also highlighted about the factors affecting the lactoferrin concentration. Although, there are several controversies regarding this factor that not only gestational age but also the length of gestation of neonates are also associated. It has also been seen that mothers of premature babies have more quantity of this protein and even after prolonged lactation it increases again.

Table 8: Co-relation between neonatal health and lactoferrin concentration:

		Lactoferrin	Presence ill health condition
Lactoferrin	Pearson Correlation	1	-.037
	Sig. (2-tailed)		.608
	N	195	195
Presence ill health condition	Pearson Correlation	-.037	1
	Sig. (2-tailed)	.608	
	N	195	195

Table 8 depicts the non-existence of any significant co-relation between ill health condition among neonates and lactoferrin concentration. A study conducted in the year 2024 by Atayde et al, ill health condition produces more lactoferrin to balance the immunity among the ill neonates. But in the present study there were only a few neonates found in ill condition.

CONCLUSION

The present study found that Maternal and neonatal parameters both positively and negatively associated with the concentration of lactoferrin present in the colostrum. The colostrum samples were collected till 4 days of post-partum. Major co-relation found between the iron and fat intake with colostrum content whereas no relation found between the carbohydrate and protein intake and hence no relation established with total energy intake. Parity of the mothers was also not associated with the concentration. Duration of gestation was found to be one of the associated factors. Ill health condition of the neonates was also not associated the lactoferrin concentration. The study also found that lactoferrin concentration decreases with the advancement of the days as its concentration decreases with the maturation of the milk. Hence it can be concluded that nutrition remains utmost important among all the factors. Iron is an important micronutrient which is crucial for the optimum lactoferrin concentration which protects both the neonates and infants from sepsis and other infection, hence reduces the mortality rate. Although more studies are required to provide more relevance regarding the concentration of colostrum in human milk.

Acknowledgement: I would like to express my heartfelt gratitude to Department of Home Science, Department of Biochemistry and Prasuti Tantra, BHU, UGC, DST-CURIE and Sir Sunderlal Hospital (BHU), and all the mothers and staff of the hospital

REFERENCES

1. Czosnykowska-Łukacka, M., Orczyk-Pawiłowicz, M., Broers, B., & Królak-Olejnik, B. (2019). Lactoferrin in human milk of prolonged lactation. Nutrients, 11(10), 2350. https://doi.org/10.3390/nu11102350
2. Sindi, A. S., Stinson, L. F., Lai, C. T., Gridneva, Z., Leghi, G. E., Netting, M. J., Wlodek, M. E., Muhlhausler, B. S., Zhou, X., Payne, M. S., & Geddes, D. T. (2024). Human milk lactoferrin and lysozyme concentrations vary in response to a dietary intervention. The Journal of Nutritional Biochemistry, 135, 109760. https://doi.org/10.1016/j.jnutbio.2024.109760
3. Yang, Z., Jiang, R., Chen, Q., Wang, J., Duan, Y., Pang, X., Jiang, S., Bi, Y., Zhang, H., Lönnerdal, B., Lai, J., Yin, S., Yang, Z., Jiang, R., Chen, Q., Wang, J., Duan, Y., Pang, X., Jiang, S., . . . Yin, S. (2018). Concentration of Lactoferrin in Human Milk and Its Variation during Lactation in Different Chinese Populations. Nutrients, 10(9), 1235. https://doi.org/10.3390/nu10091235

4. Berger, P. K., Kuncham, M., Ong, M. L., Bell, K., Lee, A. C., Sen, S., Furst, A., Bode, L., & Belfort, M. B. (2025). Associations of human milk lactoferrin with measures of physical growth in very preterm infants. Journal of Perinatology. https://doi.org/10.1038/s41372-025-02447-2
5. Turin, C. G., Zea-Vera, A., Rueda, M. S., Mercado, E., Carcamo, C. P., Zegarra, J., Bellomo, S., Cam, L., Castaneda, A., & Ochoa, T. J. (2017). Lactoferrin concentration in breast milk of mothers of low-birth-weight newborns. Journal of Perinatology, 37(5), 507–512. https://doi.org/10.1038/jp.2016.265
6. Woodman, T., Strunk, T., Patole, S., Hartmann, B., Simmer, K., & Currie, A. (2018). Effects of lactoferrin on neonatal pathogens and Bifidobacterium breve in human breast milk. PLoS ONE, 13(8), e0201819. https://doi.org/10.1371/journal.pone.0201819
7. Wang, W., An, Q., Huang, K., Dai, Y., Meng, Q., & Zhang, Y. (2024). Unlocking the power of Lactoferrin: Exploring its role in early life and its preventive potential for adult chronic diseases. Food Research International, 182, 114143. https://doi.org/10.1016/j.foodres.2024.114143
8. Villavicencio, A., Rueda, M. S., Turin, C. G., & Ochoa, T. J. (2016). Factors affecting lactoferrin concentration in human milk: how much do we know? Biochemistry and Cell Biology, 95(1), 12–21. https://doi.org/10.1139/bcb-2016-0060
9. Fujita, M., Wander, K., Ruvalcaba, N. P., & Odo, A. N. (2022). Human milk lactoferrin variation in relation to maternal inflammation and iron deficiency in northern Kenya. American Journal of Human Biology, 34(12), e23812. https://doi.org/10.1002/ajhb.23812
10. Liu, L., Guo, Q., Cui, M., Liu, J., Yang, C., Li, X., Liu, P., & Wang, L. (2021). Impact of maternal nutrition during early pregnancy and diet during lactation on lactoferrin in mature breast milk. Nutrition, 93, 111500. https://doi.org/10.1016/j.nut.2021.111500
11. Rahfiludin, M. Z., & Pangestuti, D. R. (2020). Lactoferrin Association with Maternal Nutritional Status and Lactation Stages. Current Research in Nutrition and Food Science Journal, 8(1), 174–181. https://doi.org/10.12944/crnfsj.8.1.16
12. Villavicencio, A., Rueda, M. S., Turin, C. G., & Ochoa, T. J. (2016b). Factors affecting lactoferrin concentration in human milk: how much do we know? Biochemistry and Cell Biology, 95(1), 12–21. https://doi.org/10.1139/bcb-2016-0060
13. Atayde, A. M. P., Kapoor, N. R., Cherkerzian, S., Olson, I., Andrews, C., Lee, A. C. C., Sen, S., Bode, L., George, K., Bell, K., Inder, T., & Belfort, M. B. (2024). Lactoferrin intake from maternal milk during the neonatal hospitalization and early brain development among preterm infants. Pediatric Research, 96(1), 159–164. https://doi.org/10.1038/s41390-023-03002-5.
14. Zhou, Y., Duan, Y., Jiang, S., Zhang, Y., Liu, M., Gu, X., Li, Y., Zhang, N., Jiang, R., Yang, Z., & Lai, J. (2025). Longitudinal changes in human milk lactoferrin during the first year: a prospective cohort study. Pediatric Research. https://doi.org/10.1038/s41390-025-04109-7
15. Huang, Y., Tao, Y., Yang, H., Zhang, J., Yan, B., Zhang, H., Chen, W., & Fan, D. (2025). Critical importance of iron saturation in lactoferrin: effects on biological activity, nutritional functions, and applications. Journal of Agricultural and Food Chemistry, 73(18), 10665–10680. https://doi.org/10.1021/acs.jafc.4c11535

